IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Klein *et al.*

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For: METHODS OF CREATING CONSTRUCTS USEFUL FOR INTRODUCING SEQUENCES INTO EMBRYONIC STEM CELLS

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
BOX PATENT APPLICATION
Washington, D.C. 20231

Sir:

Prior to examination of the above-referenced application, entry of the following amendment is respectfully requested.

In the Claims:

Please cancel claims 1-50.

Please add new claims 51-61 as follows:

51. (New) A method of producing a targeting construct, the method comprising:

(a) amplifying a polynucleotide sequence comprising a first region homologous to a target gene or sequence and a second region homologous to a target gene or sequence;

(b) providing a positive selection marker; and

(c) annealing the positive selection marker with the polynucleotide sequence to form the construct using ligation independent cloning, such that the positive selection marker is positioned between the first region and second region of the target gene or sequence;

wherein the first region and second region homologously recombine with the target gene or sequence.

52. (New) The method of claim 51, wherein the polynucleotide sequence is amplified directly from a plasmid library.

53. (New) The method of claim 51, wherein the positive selection marker is a neomycin resistance gene.

54. (New) The method of claim 51, wherein the polynucleotide sequence is amplified with oligonucleotide primers that are at least 12 nucleotides in length and have a 5' sequence lacking one type of base.

55. (New) The method of claim 51, wherein the construct further comprises a screening marker.

56. (New) The method of claim 55, wherein the screening marker is a fluorescent protein.

57. (New) A method of producing a targeting construct, the method comprising:

(a) amplifying a first polynucleotide sequence corresponding to a target gene or sequence from a circular plasmid library, wherein the polynucleotide sequence comprises first and second regions which are homologous to the target gene or sequence; and

(b) inserting a second polynucleotide sequence encoding a positive selection marker between the first region and second region to form the construct using ligation independent cloning,

wherein the first region and second region homologously recombine with the target gene or sequence.

58. (New) The method of claim 57, wherein the positive selection marker is a neomycin resistance gene.

59. (New) The method of claim 57, wherein the polynucleotide sequence is amplified with oligonucleotide primers that are at least 12 nucleotides in length and have a 5' sequence lacking one type of base.

60. (New) The method of claim 57, wherein the construct further comprises a screening marker.

61. (New) The method of claim 60, wherein the screening marker is a fluorescent protein.

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Remarks

Claims 51-61 are newly added. New claims 51-61 do not introduce new matter to the subject application and are fully supported throughout the application. Upon entry of the above amendment, claims 51-61 are pending in the instant application. Examination on the merits of the application is respectfully requested.

Respectfully submitted,
DELTAGEN, INC.

02/28/02
Date

Mariette A. Lapiz

740 Bay Road
Redwood City, CA 94063

Mariette A. Lapiz
Reg. No. 44,202
Phone: 650/569-5172